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Pain evaluation in dairy cattle

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ABSTRACT

Pain compromises the welfare of animals. A prerequisite for being able to alleviate pain is that we are able to recognize it. Potential behavioural signs of pain were investigated for dairy cattle with the aim of constructing a pain scale for use under production conditions. Forty-three cows were selected and fifteen different behaviours were scored, subsequently a clinical examination was performed to allocate the cows to a pain and non-pain group. The animals were then treated with an analgesic or a placebo and after a resting period the cows were re-scored by two observers blinded to the treatment. Six behaviours were found to be significantly different between the pain and non-pain group and robust enough to be included in the pain scale: 'attention towards the surroundings' 'head position', 'ears position', 'facial expressions', 'response to approach' and 'back position' (a seventh, piloerection, was also significant but seemed difficult to use as it changed rapidly; $p < 0.05$ for all measures). The Cow Pain Scale is the sum of the score for the aforementioned behaviours. For each individual animal before and after treatment, it was significantly lower after analgesic treatment ($p = 0.003$) in the ClinPain group but not after placebo treatment ($p = 0.06$); the pain score did not differ significantly before compared to after treatment with analgesic or placebo for the non-pain group ($p = 0.2$; $p = 0.1$). A second study was conducted to further validate the Cow Pain Scale. Cows from two herds were randomly selected ($n = 119$) and their behaviour scored by two observers. Subsequently the cows were clinically examined and allocated to a pain and non-pain group ($n = 96$, 23 cows were excluded because of incomplete examination). The cows from the pain group scored higher on The Cow Pain Scale compared to the non-pain group for both observer I ($p < 0.0001$) and observer II ($p = 0.0001$). For the two observers the sensitivity of the Cow Pain Scale was calculated to 0.61/0.75 and the specificity to 0.75/0.75 with a weighted Kappa of 0.62. In conclusion the Cow Pain Scale has the potential to be applied for the assessment of pain in dairy cattle under production conditions.

1. Introduction

Pain is an important animal welfare problem, not least in cattle (Huxley and Whay, 2006; Hewson et al., 2007; Kielland et al., 2009; Laven et al., 2009; Thomsen et al., 2010; Fajt et al., 2011). Veterinarians are expected to be able to diagnose, grade and treat pain in cattle. Large differences in analgesic treatment practices are related to age and gender of the veterinarian but also attributed to cost and availability of analgesics (Huxley and Whay, 2006). One reason for the inconsistence of pain relief for cattle is the inadequate ability to assess pain (Flecknell, 2008). Pain assessment based on physiological parameters has proven inapplicable as these are often unspecific and sensitive to stress as well as being difficult to measure on-farm (Hansen, 1997). Therefore, pain assessment based on behaviour has received increasing attention as this principle has been applied to assessment in Nellore cattle after castration and in several other species (Holton et al., 2001; Pritchett et al., 2003). Three classes of behaviours, useful for pain evaluation of animals, have been proposed (Weary et al., 2006): (1) pain specific behaviours, (2) a change in certain behaviours that the animals are very motivated to perform (e.g. feeding) and (3) preference choices. While preference choices are suitable for research purposes, pain specific behaviours and to a lesser extent the change in certain normal behaviours are more practically useful. However, the change in normal behaviours is not a readily usable measure as it necessitates long observation times.

Pain specific bovine behaviours described in veterinary textbooks are often behaviours that are linked to diseases believed to

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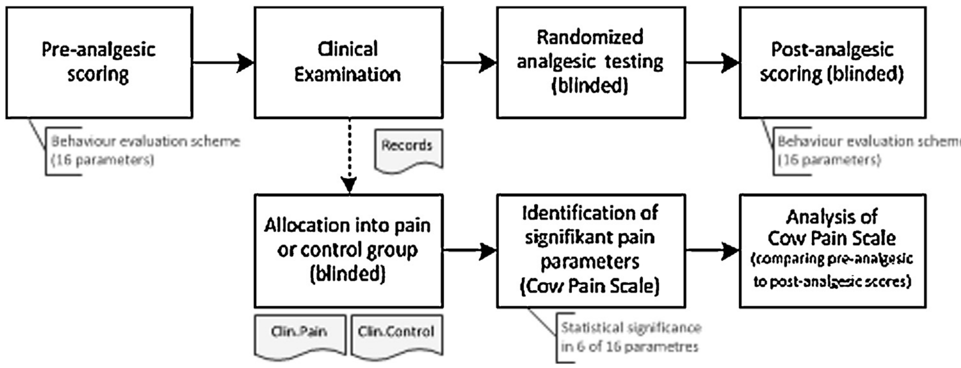

Fig. 1. Flow diagram of study I.

be extremely painful, such as acute toxic mastitis, fractures, septic arthritis and peritonitis (Huxley and Whay, 2006). These pain behaviours comprise: changed posture (crouching, arched back, low head position), severe lameness, attention towards the painful area, vocalization, teeth grinding (bruxism), and modification of social behaviour (Sanford et al., 1986; Short, 1999; O'Callaghan et al., 2003; Sandem et al., 2006; Radostits et al., 2007; Hudson et al., 2008; Chapinal et al., 2010; Leslie and Petersson-Wolfe, 2012). The behaviours range from obvious to subtle but occurrence, grading or co-existence with diagnoses has never been established. Cattle are often described as stoic, i.e. they do not display obvious pain behaviour. However, during the last decade, research in a number of other supposedly stoic prey species, e.g. horses (Dalla Costa et al., 2014; Gleerup et al., 2015), rats (Sotocinal et al., 2011), mice (Langford et al., 2010) and rabbits (Keating et al., 2012), have shown that subtle changes in behaviour are good predictors of pain, among these facial expressions (Leach et al., 2012). To the knowledge of the authors facial expressions of pain in cattle have not been described in detail but considering recent research within this field, it is highly likely that similar facial cues of pain exist in cattle.

The overall aim of this study was to identify possible pain-specific behaviours in dairy cattle and to combine these into a practically useful pain scoring tool. The focus of the study is on pain behaviours that are exhibited by dairy cattle under commercial conditions. The specific aims of the study were (1) to construct a pain scale by investigating the occurrence of behaviours expected to be related to pain in cows with and without pain and subjected to analgesic or placebo treatment (study I), and (2) to investigate the practical performance of this pain scale in randomly selected cows with different observers (study II).

2. Study I

To confirm suspected pain, analgesic testing is a gold standard method (Weary et al., 2006). If a given specific clinical sign of pain is reduced or eliminated after the analgesic treatment, the animal was most likely to have been experiencing pain before the treatment. This type of analgesic testing has good specificity but poor sensitivity as absence of effect may be caused by inefficiency of the chosen analgesic on certain types of pain, rather than the sign was not caused by pain. In this study, analgesic testing was employed and selected behaviours were scored before and after treatment. Cows were selected on day 1 and behaviour was scored (afternoon) according to selected behavioural parameters. On day 2, the cows were subjected to a clinical examination and then treated with an analgesic or a placebo. After a resting period, a second behaviour score was performed (afternoon). Post hoc, the cows were divided into a pain group (ClinPain) and a placebo group (ClinPlac) based on the findings of the clinical examination (for an outline of the study, see Fig. 1).

2.1. Animals, materials and methods

The experimental protocol was approved by the Danish Animal Experiments Inspectorate.

2.1.1. Herds

Three herds of >150 Danish Holstein dairy cows, loose housed on slatted floors were included in the study. All herds had a monthly advisory consultancy with a veterinarian, following Danish legislation. The herds were collected as convenience sampling.

2.1.2. Animals

Inclusion criteria were: lactating cows >2 weeks after calving with no veterinary diagnosis. As many cows as possible were examined in the herds within the study period; approximately 10–12 cows per day. Fifty cows were included but to be able to study pain behaviour as opposed to sickness behaviour two cows were excluded post hoc due to rectal temperature >39.2 °C). An additional five cows were excluded due to lack of claw examination. Forty-three cows were included in the study.

2.1.3. Behaviour evaluation scheme

The behaviour evaluation was based on pain behaviours selected from the literature (Morton and Griffiths, 1985; Sanford et al., 1986; Short, 1999; O'Callaghan et al., 2003; Sandem et al., 2006; Radostits et al., 2007; Hudson et al., 2008; Chapinal et al., 2010; Leslie and Petersson-Wolfe, 2012). The behaviours included in the behaviour evaluation scheme is described in detail in Table 1. All behaviours were weighted and graduated in 3–5 levels (see Supplementary material table X) as some behaviours are considered more pain specific than others and therefore should be more weighty in the final pain score sum (Gleerup and Lindegaard 2015). Specifications of the 'bovine pain face' and ear positions (Fig. 2a and b) were modelled after the Equine Pain Face (Gleerup et al., 2015), modified by the information from observing six healthy experimental cows before and after analgesic treatment following a standard rumen fistulation surgery. These observations were performed by the first author, who was already trained in the evaluation of the Equine Pain Face. Lameness is traditionally used as an indicator of orthopaedic pain but was excluded from the list of investigated pain behaviours, since it was included in the clinical examination and thus used to validate the behaviours in Table 1.

2.1.4. Behavioural and clinical examination

Only cows in cubicles or walking areas were included. To increase the probability of including a balanced number of cows with and without pain, cows were selected and temporarily allocated into two groups, based on a visual inspection from the distance. This inspection discriminated between sound looking cows (TempContr), that were bright and alert and cows with an

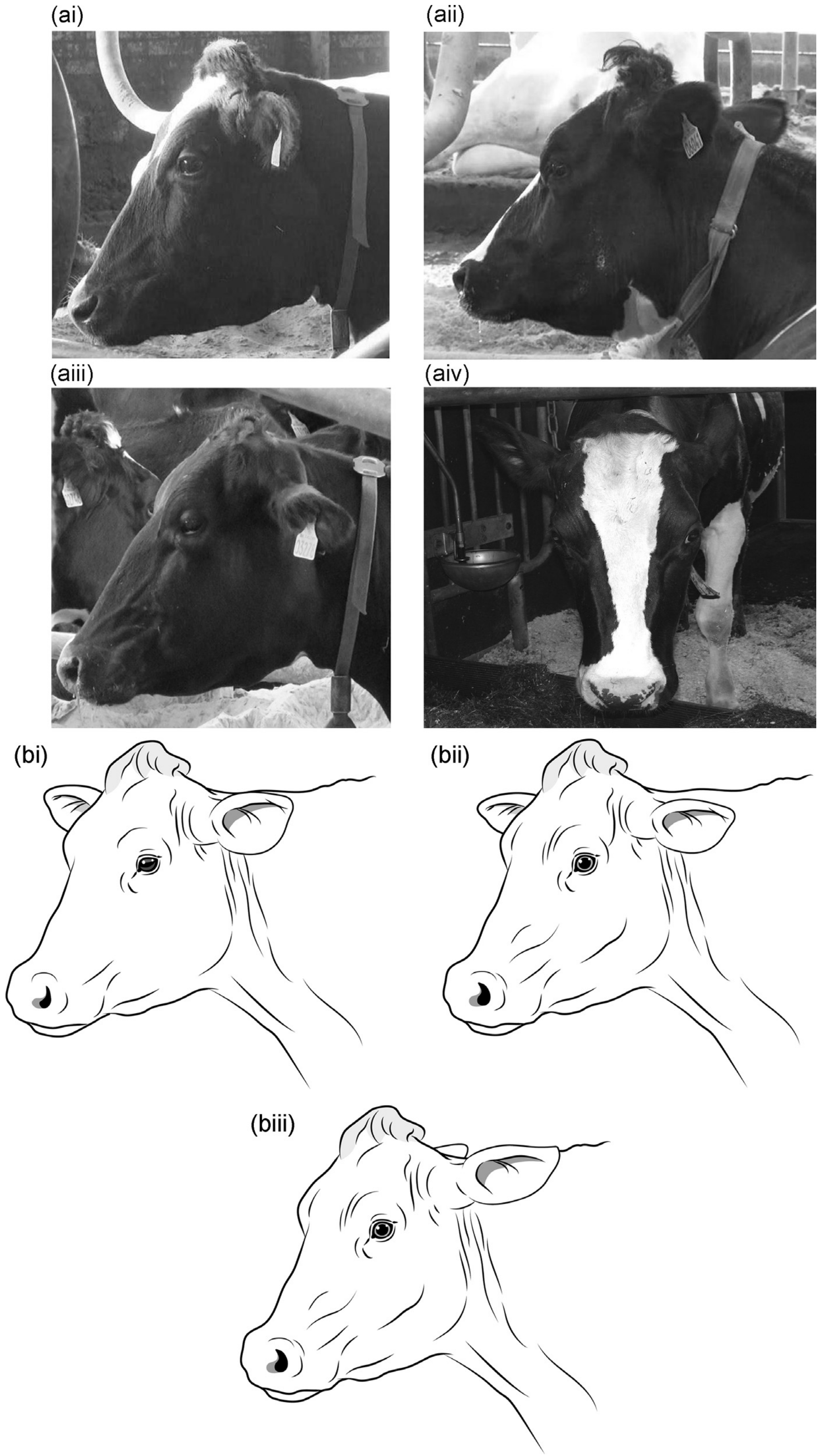

Fig. 2. (a) Photos of a cow relaxing, not in pain (I) and three cows in pain: lameness (II), compromised vascular system, udder sore, few and week peristaltic movements (III) and post-surgical pain after rumen fistulation (IV). The features of the pain face of the cow comprise changes in 4 areas: (1) Ears: ears are tense and backwards (II) or low/lambs ears (III). (2) Eyes: eyes have a tense stare (II + IV) or a withdrawn appearance (III). Tension of the muscles above the eyes may be seen as 'furrow lines' (III + IV). (3) Facial muscles: tension of the facial muscles on the side of the head (II + III). (4) Muzzle: strained nostrils, the nostrils may be dilated and there may be 'lines' above the nostrils. There is increased tonus of the lips (II + III + IV). (b) Illustrations of the Cow Pain Face. The scientific illustrations aim at accentuating the important changes in the facial expression without disturbances of the specific cow's individual expression. (I) Relaxed cow. (II) Cow in pain with low ears/lambs ears. (III) Cow in pain with ears tense and backwards. Illustrations Anders Rådén.

unsound appearance (TempCase) due to a dull fur coat, shallow respiration or other irregularities that were visible from a distance of approximately 20 m. The initial grouping was based on this cursory inspection whereas the later and formal grouping was performed post hoc, based on the findings of a clinical examination. Immediately after selecting each cow, two veterinary observers performed the behavioural evaluation. The evaluation started at a distance by observing the undisturbed behaviour of the cow and proceeded with approaching the cow to evaluate the "response to approach" and if the cow was lying down, encouraging it to stand or walk for evaluating "head position", back position and lameness (lameness as a part of the clinical examination). The behavioural observations were performed independently and blinded between the two observers.

2.1.5. Clinical examination

The morning following the first behavioural evaluation, the cows from the groups TempContr and TempCase were separated from the herd for a full clinical examination. The cows remained separated from the herd for 1–3 h and they were tethered for a part of that time. All cows were randomly allocated to one of two treatments: the non-steroidal anti-inflammatory drug ketoprofen 150 mg/ml or a placebo treatment with saline. Ketoprofen was chosen as it is commonly used analgesic for cattle. Half of the cows from each group (TempContr and TempCase) were treated with analgesia and the other half with saline. The treatments were given as intravenous injections of 12 ml and were randomized and blinded to the observers. After examination and treatment, the cows were marked for recognition and were reintroduced to the herd where they were allowed to rest for 2–4 h. The two observers then performed a second behavioural evaluation, following the same procedure as the first evaluation. The cows were recognized using the markings and the second evaluation was performed without considering the first evaluation or temporary grouping. A claw examination and trimming according to need was performed on all cows 2–5 days after the clinical examination. Postponing the claw examination relative to the clinical examination was necessary due to logistics in the herds. The claw examination was performed in a claw box by the usual herd claw trimmer; findings were confirmed by the veterinary observers and added to the clinical case record for each cow.

2.2. Statistical analysis and development of the Cow Pain Scale

The grouping of the animals for the statistical analyses was performed post hoc, and was based solely on the results of the clinical examination and independent of the temporary grouping and the results of the behavioural scores. Clinical findings of conditions regarded as painful (Table 2) were used to allocate cows to the pain group (ClinPain) whereas cows without these clinical findings were allocated to the control group (ClinContr). The composition of groups TempCase and TempContr were compared with the composition of the groups ClinPain and ClinContr.

For each behavioural indicator, differences between the ClinPain group and the ClinContr group were tested using the Mann–Whitney test. A p-value ≤ 0.05 was considered significant. Behavioural indicators where scores differed significantly between the two groups were included in a pain scoring scheme, henceforth called "the Cow Pain Scale". The total score of the Cow Pain Scale was compared within and between the ClinPain and the ClinContr groups, before and after analgesic or placebo treatment. The Mann–Whitney test was used for within group testing and the Wilcoxon matched-pairs signed rank test was used for between groups testing, with p-values ≤ 0.05 as significant.

2.3. Results

Forty-three cows were included in the statistical analysis. The comparison of the groups TempCase and TempContr with the groups ClinPain and ClinContr revealed that three cows changed status: two cows from the group TempCase was allocated to the ClinContr group and one cow from the group TempContr was allocated to the group ClinPain. Six of the 15 tested specific behaviours potentially indicating pain were never observed for any of the cows and therefore not included in the Cow Pain Scale; these were: chewing, tooth grinding, moaning, shivering, tenesmus (abdominal straining with little production of either faeces or urine) and weight shifting/kicking. Of the remaining nine potentially pain specific behaviours, the score of seven were significantly higher in the ClinPain group than in the ClinContr group (Table 3). The behaviour 'piloerection' was excluded from the Cow Pain Scale as the observers found this parameter difficult to evaluate because it changed rapidly. A total of six parameters were therefore included in the Cow Pain Scale. The descriptions of each level of the parameters were re-evaluated for usability and any two levels (descriptions) that were estimated to be difficult to distinguish from one another were collapsed to one level. This resulted in a pain scale with six parameters, each described in two or three levels (Table 4). The sum of the Cow Pain Scale was significantly higher ($p < 0.0001$) for the ClinPain group compared to the ClinContr group (Fig. 3). The pain scores are grouped out on either side of 'score 3', indicating that cows with a score higher than 'score 3' are likely to be in pain. Accordingly, 'score 3' is suggested as the cut-off value for the Cow Pain Scale. Furthermore, when comparing the sum of the Cow Pain Scale for each individual animal before and after treatment, it was significantly lower after analgesic treatment ($p = 0.003$) in the ClinPain group (group median 7, interquartile 4.25 to 8; group median 5, interquartile 2.25 to 6) but did not change significantly after placebo treatment ($p = 0.06$, median (before) 6, interquartile 4 to 9; median (after) 3, interquartile 2 to 6). For the ClinContr group, there was no significant difference between the total pain score before and after treatment with analgesic or placebo ($p = 0.2$; $p = 0.1$).

3. Study II. Practical performance of the Cow Pain Scale

The purpose of this study was to validate the Cow Pain Scale. Study I concluded that the pain scores for the cows with clinical signs of pain were higher than the pain scores for the cows without clinical signs of pain and that treatment with analgesia decreased the pain score in the cows with clinical signs of pain. Study II focused on the assessment of the specificity, sensitivity and practical performance of the Cow Pain Scale.

3.1. Materials and methods

3.1.1. Herds

Two herds with Danish Holstein Friesian dairy cows were included. One herd was also included in study I however the sampling for study II took place in another new barn12 months after the sampling for study I. All cows were loose housed on concrete floor with cubicles.

3.1.2. Animals

Animals were selected by random sampling. This was attained by selecting the cow standing or lying in every fifth cubicle, alternating between the left and the right sides of all the aisles of the barns with the lactating cows. Cows standing in the walking area were not selected as they could not be observed undisturbed. Each cow was scored immediately following selection. The scoring was

Table 1
Description of behaviours evaluated in the behaviour evaluation scheme.

Category	Definitions of behaviours
Attention	Is the cow attentive towards the surroundings? Is the cow active, performing normal cow activities such as eating, ruminating or sleeping? Is the cow facing the wall/away from conspecifics or is the cow relaxed and following activities in the near surroundings? 'Attention' should be evaluated when the cow is undisturbed
Head bearing	The head bearing is evaluated as being below withers, at withers or above withers. The head position may be evaluated when the cow is standing, walking or lying down (not sleeping)
Ear position	The ears on a relaxed cow may be positioned forward or frequently moving while a cow in pain may have low ears or both ears consistently backwards (see Fig. 2a and b). 'Ear position' should be evaluated when the cow is undisturbed
Facial expression	Changes in muscle tension along the sides of the head and above the eyes manifested as oblique lines or above the nostrils manifested as wrinkles should be noted. The nostrils may be dilated (see Fig. 2a and b for further details). 'Facial expression' should be evaluated when the cow is undisturbed
Eye white (visible)	The proportion of white visible in the eyes of the cow
Nostril cleanliness	Evaluation of the presence of nasal discharge and of whether the action of cleaning the nostrils has been observed. Dust or sand on the muzzle is not considered a lack of nostril cleanliness
Chewing	Chewing without feed in the mouth
Tooth grinding	Pressing the teeth hard together, resulting in a creaking sound
Vocalizing	Moaning or grunting, usually on expiration
Shivering	Muscle tremors
Tenesmus	Abdominal straining with little production of either faeces or urine
Piloerection	Erect hair on the neck and back
Response to approach	The response elicited when approaching the cow slowly with one hand kept in the level of the observer's waist, reaching towards the cow
Back position	The contour of the top line of the standing or walking cow
Weight shifting	Frequent unprovoked stepping and kicking with the hind limbs

Table 2
Clinical findings that were used to allocate the cows to the ClinPain group (study I) and the PAIN group (study II). Each cow could have one or several of these findings (% indicates the fraction of cows in the pain group with the specific clinical finding). This list of potentially painful clinical findings reflects the disease pattern of the herds included in this study. The disease pattern differed between the herds.

Organ system clinical findings—potentially painful		Study I ($n = 23$)	Study II ($n = 41$)
Lameness	Very lame (degree 2 of 0-1-2) No or minimal weight bearing on the affected leg or low degree lameness on more than one leg	12 (52%)	13 (32%)
Circulatory	Compromised circulation (capillary refill time (crt) increased >4 s, dehydration or edema, presumably not painful but may be caused by some painful disorder)	4 (17%)	8 (20%)
Gastrointestinal system	Tympanic sounds from the gastrointestinal system (right side or very obvious left side) or pinched with tense abdominal muscles	3 (13%)	9 (23%)
Respiratory system	Respiratory disease with nasal discharge (seromucous), wheezes, bronchus respiration or forced respiration	3 (13%)	3 (7%)
Genitalia	Internal lazeration	2 (9%)	0
Integument	Wounds/contusions (of severe character or multiple) (udder sores are categorized under 'udder')	14 (61%)	1 (2%)
Udder	Mastitis, inflammation, painful at palpation or hard/tense udder	1 (4%)	20 (49%)
	Udder sores	0	16 (39%)
Ketosis	Severe (presumably not painful but may be caused by some painful disorder)	1 (4%)	0
Claws	Severe toe ulcers, sole ulcers or digital dermatitis	8 (35%)	–
Fractures	Tentative diagnose, diagnosed only by palpation and conformation changes (coxae and pelvis)	2 (9%)	0

Table 3
p-Values for each of the specific behavioural parameters from study I. Comparison of the pain group and the control group. Statistical significance was set at $p < 0.05$. Percentiles (25% and 75%) are listed.

Behavioural parameter	ClinContr	Percentiles		ClinPain	Percentiles		p Value
	Median	25%	75%	Median	25%	75%	
Attention	0	0	0	2	0	2	<0.0001
Head position	0	0	0.75	2	1	3	<0.0001
Ear position/movement	0	0	1	2	2	4	<0.0001
Facial expression	0	0	0	2	2	2	<0.0001
Visible eye white	0	0	1	0.5	0	1	0.8
Nostril cleanliness	1	1	2	1	1	2	0.5
Piloerection	0	0	1	1	0	2	0.047
Response to approach	0	0	1	2	1	2	0.0008
Back position	0	0	0.75	2	1	2	<0.0001

performed independently and blinded by two observers according to the Cow Pain Scale (Table 4). A total of 119 cows were scored.

3.1.3. *Clinical examination*

The clinical examination was performed by the two observers. A lameness evaluation was performed in relation to the pain scoring whenever possible, while the remaining clinical examination was performed in the afternoon or the morning following the pain scoring session. In contrast to study I, a full examination of the claws was not performed. The cows were separated from the herd for the clinical examination. The separation procedure was unsuccessful for a number of cows, leaving 96 cows with both a clinical examination and a complete Cow Pain Scale score from both observers.

Table 4
The Cow Pain Scale including the pain specific behaviours.

Score	0	1	2
Attention towards the surroundings	Active and attentive The cow is active: eating, ruminating, grooming etc. The cow is attentive and/or attention seeking/curious	Quiet/depressed The cow is not active, avoiding eye contact, may move away from the observer	
Head position	High/level of withers The cow is active, eating, ruminating or is contact seeking/curious	Level of withers The cow is *not active*, not eating, ruminating, grooming or sleeping	Low The cow is *not active*, not eating, ruminating, grooming or sleeping; may lie down quickly after getting up
Ear position	Both ears forward *or* one ear forward or back and the other listening	Ears back/asymmetric ear movements Both ears back or moving in different directions (not forward or back)	Lambs' ears Both ears to the sides and lower than usual; the pinna facing slightly down
Facial expression	Attentive/neutral look The cow is attentive, focused on a task (eating, ruminating) or sleeping	Tense expression/strained appearance The cow has a worried or strained look, furrows above the eyes and puckers above the nostrils	
Response to approach	Look at observer, head up, ears forward or occupied with activity (grooming, ruminating)	Look at observer, ears *not* forward, leave when approached	May/may *not* look at observer, head low, ears *not* forward may leave slowly
Back position	Normal	Slightly arched back	Arched back

3.1.4. Observers

Observer I was a sixth year veterinary student and observer II was a veterinarian with two years of experience from cattle practice. The observers were introduced to the Cow Pain Scale theoretically using pictures and video footage. Prior to the study period the observers were educated with a practical session in one of the study herds, given by the first author. The total instruction time was approximately 4 h.

3.2. Statistical analysis

For the statistical analysis the 96 included cows were divided into two groups, a PAIN group ($n = 41$), and a CONTROL group ($n = 55$), based on the clinical examination. Cows were allocated to the PAIN group if they had one or more of the clinical findings described in Table 2. All cows in the CONTROL group were free from any of the clinical findings listed in Table 2. The statistical analysis was performed by comparing the Cow Pain Scale of cows in the PAIN group with cows in the CONTROL group. Data distribution was assessed using the D'Agostino and Pearson omnibus normality test. The statistical analysis was carried out as a one-tailed t-test with Welch correction. Statistical significance was set at $p < 0.05$. The statistical package GraphPad Prism version 6.05 (GraphPad Software Inc., La Jolla, CA, USA) was used. The sensitivity and specificity was calculated for both observers. The inter-observer agreement between the two observers was evaluated by weighted Kappa calculations using GraphPad QuickCalcs (GraphPad Software Inc., La Jolla, CA, USA) for analysis of categorical data. For the graphical presentation of the agreement between observers, random noise (between 0 and 0.1) was added to data for improved visualization of all data points in a scatter plot. This was carried out using the RAND function in Microsoft Excel 2010.

3.3. Results

The pain scores were significantly higher for cows in the PAIN group compared to cows in the CONTROL group for both observer I ($p < 0.0001$) and observer II ($p = 0.0001$) (Fig. 4). In study I 'score 3' was suggested as the cut-off value, indicating that a pain score above 'score 3' was indicative of pain. Using this cut-off value, the sensitivity and specificity of the pain score, calculated from a 2×2 table resulting in: for the inexperienced observer I 0.61 (95% CI, [0.45, 0.75]) and 0.75 (95% CI, [0.0, 61, 0.85]) respectively and for the experienced observer II 0.76 (95% CI, [0.59, 0.87]) and 0.75 (95% CI, [0.0, 61, 0.85]) respectively. The inter-observer agreement between observer I and observer II, the weighted Kappa coefficient K_W, was 0.62, which shows substantial agreement (Landis and Koch, 1977).

4. Discussion

Most of the pain behaviours investigated in this study were selected on the basis of common knowledge, from veterinary textbooks and published papers on cattle diseases. Several of the pain behaviours in the initial list were never observed during the study, possibly because some of the pain behaviours, e.g. vocalization, only have been reported for severe pain, (Morton and Griffiths, 1985; Hansen, 1997). The results of study I, suggest six subtle behavioural signs of pain that are useful for pain evaluation in dairy cattle. Unified in the Cow Pain Scale they showed a relatively high sensitivity and specificity - a high pain score predicting a high probability of being in pain as assessed by a clinical examination. Furthermore, the Cow Pain Scale showed substantial inter-observer agreement between the two observers. The sensitivity and specificity was calculated by applying a cut-off value of 3. The pain scores of the pain and control groups dispersed relatively close to the cut-off value in study II, which may be explained by the selection procedure in which test animals were randomly selected from a population of sound, lactating cows. Cows with acute severe pain were assumed to be in treatment and were not included in the study. A distribution of animals according to their severity of pain with most of the animals feeling some, but not severe pain would yield the present result.

The behavioural parameters that were included in the Cow Pain Scale were similar to those described for other species: the changed attention in horses (Pritchett et al., 2003; Gleerup et al., 2015), lowered head position (Taylor et al., 2002; Price et al., 2003; Lindegaard et al., 2010), changed ear positions/lowering of the ears (Langford et al., 2010; Sotocinal et al., 2011), altered facial expressions (Langford et al., 2010; Sotocinal et al., 2011; Keating et al., 2012), altered response to approach (Pritchett et al., 2003) and back arching (Langford et al., 2010; Sotocinal et al., 2011; Keating et al., 2012). For the facial expressions, there are also substantial similarities with horses: the low ears, the tension of the muscles alongside the head (mimic muscles and chewing muscles), the dilated nostrils, the tense stare and the tension above the eyes (Dalla Costa et al., 2014; Gleerup et al., 2015). The changed attention towards the surroundings and the lowered head and back arching was also found in Nellore cattle after castration (de Oliveira et al., 2014). However, this scale did not include changes in ear position, facial expressions and response to approach.

This study included a pain group and a control group, both divided into groups of analgesic and placebo treatments. This is considered to the best possible method in clinical pain studies (Weary et al., 2006). The lack of an effect of the analgesic treatment in the control group in study I suggests that there was no generally inhibitory or excitatory effect of the analgesic drug, ascertaining that the reduced pain score in the ClinPain group could be ascribed to the pain-relieving effect of the analgesic drug. The analgesic treatment chosen for this study was ketoprofen, an NSAID approved for anti-inflammatory and anti-pyretic indications. Dairy cattle may experience both acute and chronic pain originating from somatic or visceral structures. High-risk areas for injury, inflammation and consequently pain in dairy cattle are the udder, the reproductive organs and integuments and claws. Ketoprofen reduces inflammation and alleviates acute pain whereas chronic pain was most likely not affected. Chronic pain has not been investigated much in cattle but research suggests the presence of chronic pain in calves after castration (Molony and Kent, 1997) and in heifers after tail docking (Eicher et al., 2006). Chronic pain following laminitis and chronic lameness in horses has been described (Driessen et al., 2010) possibly comparable to claw lesions and other orthopaedic injuries in cattle. Nonetheless, if chronic pain has an inflammatory component, ketoprofen may have slightly reduced it.

A complete clinical examination was chosen to categorise animals into pain and control groups. An obvious shortcoming of clinical diagnosis is that it is not directly related to pain. However, it is currently the only measure used for deciding on the need for analgesic treatment in cattle. In study I, an examination of the claws was included in the clinical examination. The cows with severe claw lesions very often had severe lameness. No cows were allocated to the pain group based on the claw examination alone. The claw examination was not included in study II which reduced animal stress and allowed for a larger number of animals to be included in the study.

In study I, the grouping of cows based on the clinical findings closely resembled the initial temporary groups which were based only on a cursory inspection. Obviously this observation method is highly dependent on the skill of the observer and therefore difficult to standardize. This initial and selection of cows based on visual inspection was employed to improve the chances of actually including some animals in pain as we had no previous experience with the prevalence of painful cows in a commercial dairy herd. The method can be argued to have affected the first behavioural scoring of the cows. On the other hand, animals that obviously stand out from the group when observed from the distance would always deviate from the normal, even if randomly sampled. We found it impossible to avoid bias from a first-hand impression affecting judgement. The subsequent blinding of the analgesic treatment and the blinding of the second behavioural scoring was therefore essential to this study.

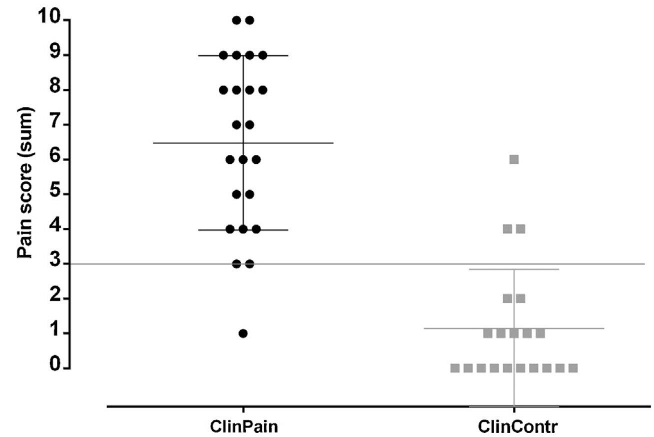

Fig. 3. The sum of the pain score for all cows in both groups, the suggested cut-off value is indicated by a line (study I).

The Cow Pain Scale seemed to be applicable for herds with a management system like the most common Danish system which produces relatively fearless cows. In a production system where the cows are not used to being handled or are used to rough handling, they will be more timid (Hemsworth et al., 2002) which will affect their response to approach and make evaluation of the undisturbed behaviour difficult thereby reducing the sensitivity of the Cow Pain Scale. Other factors like disease pattern, prevalence of acute and chronic pain, age distribution and other production related factors

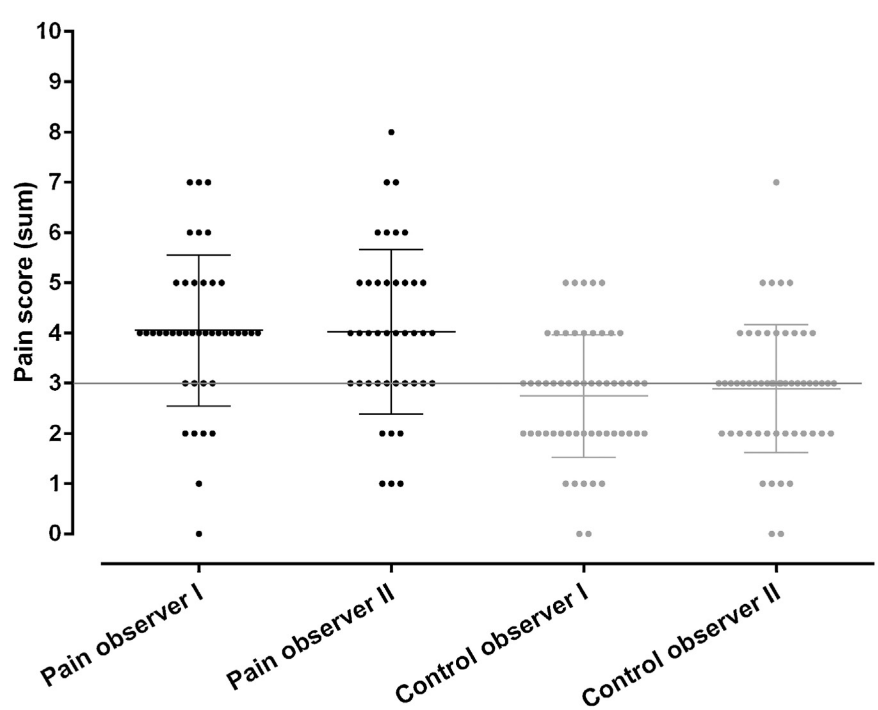

Fig. 4. Distribution of pain scores for both observers and both groups, the suggested cut-off value is indicated by a line (study II).

may influence the sensitivity and specificity of the pain scoring. A noticeable fraction (43%) of the randomly selected cows in study II was allocated to the pain group. The study II herds were considered well managed with new barns including cubicles which met the newest standards for size and bedding material. Nevertheless, there was a high prevalence of severe lameness in the sample which is in agreement with previous reports (Otten et al., 2013). In this study, the lameness evaluation was included in the clinical examination and therefore it was not included in the Cow Pain Scale. However, for future use the Cow Pain Scale could be further amplified by adding the 'lameness' score, which is also one of the scores that is seen to increase after castration in a study with Nellore cattle (de Oliveira et al., 2014).

We suggest that the Cow Pain Scale may become useful as a screening tool in a herd and possibly also for repeated observations of animals receiving analgesics to evaluate treatment effect and rehabilitation. The utility of the scale for animals in severe pain has not yet been investigated. The utility for severe pain might improve from adding a score of 'gross pain behaviour' (e.g. tooth grinding and vocalization) as this could comprise several of the classical pain behaviours that were not observed in this study. Furthermore, validation is needed for cows with fever, as fever is known to cause behavioural changes (Millman, 2007).

5. Conclusions

In conclusion, the present study showed that animals used to handling, with clinical diagnoses that may be painful had a significantly higher pain score on the Cow Pain Scale than cows in a healthy control group. The Cow Pain Scale included, 'attention towards the surroundings', 'head position', 'ears position', 'facial expressions', 'response to approach' and 'back position'. Furthermore, treatment with a systemic analgesic significantly reduced the pain score of the group, where clinical examination suggested pain but did not affect the cows in the control group. Taken together these results suggest that the Cow Pain Scale may be used to identify cows in pain.

Conflict of interest statement

There is no conflict of interest in this study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.applanim.2015.08.023.

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